ENVIRONMENTAL ASSESSMENT OF AN ACTIVE OIL FIELD IN THE NORTHWESTERN GULF OF MEXICO, 1977-1978

VOLUME II - DATA MANAGEMENT AND BIOLOGICAL INVESTIGATIONS

A report to the Environmental Protection Agency on work conducted under provisions of Interagency Agreement EPA-IAG-D5-E693-EO During 1977-1978

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ABSTRACT

Brown (Penaeus aztecus) and white (P. setiferus) shrimp of several sizes were exposed to concentrations of Buccaneer Oilfield produced brine effluents ranging from 1,000 to 500,000 ppm (v/v) in 96-hour static bioassays. LD50's ranged from 3,000 to >10,000 ppm during the months of the test (August 1977 through March 1978). Long-term tests with feeds soaked in effluents suggested that such exposure might predispose to stress in subsequent acute exposure tests.

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Volume I is designed to be used as a briefing document and as a key to more detailed scientific and technical information contained in Volumes II and III. Objectives, methods and results for each work unit are summarized in greatly abbreviated form within Volume I to facilitate dissemination of information. Thus, Volume I can be used alone or as a reference to companion Volumes II and III. Complete citations for literature cited in Volume I can be found in the Volumes II or III in which the detailed work unit reports are presented.

It is hoped that such an approach to environmental impact information dissemination will make the Annual Report a more useful and widely read document.

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INTRODUCTION

This study was started in the summer 1977 to provide laboratory information complementing field observations of the possible toxicity of Buccaneer Oilfield produced brine effluents. Experiments were conducted with two species of penaeid shrimp native to the Gulf of Mexico, the white shrimp, (Penaeus setiferus) and the brown shrimp (P. aztecus). Both species play an important part in the fisheries industry and hence the economy of the Gulf area. Since the adult and larval phases of the life cycle of these animals takes place in the offshore regions of the Gulf, both short-term and long-term effects of produced brine effluents would be of interest.

The objectives of this study were three-fold: (1) to determine the 96-hour survival of penaeid shrimp exposed to varying concentrations of Buccaneer Oilfield effluents; (2) to determine whether there were differences in response to the effluent with season, with size of test animal, and with species of test animal; and (3) to attempt to demonstrate long-term effects of effluents upon penaeids.

EFFLUENT SAMPLES

The study area and the platforms from which the produced brine effluents were obtained have been described by other investigators (Harper, D. E., Jr., 1978). Effluent samples were obtained from both Platforms 288-A and 296-B from July through October of 1977. Subsequent samples (November 1977 through February 1978) came solely from Platform 296-B following changes in operational procedures by Shell Oil Company.

Effluent was collected in clean glass bottles of about 2-l capacity, covered with aluminum foil and fitted with a tight cap. Whenever possible, samples were refrigerated after collection until returned to the laboratory, where samples were either refrigerated or frozen until testing. A single initial sample of about 20-l of effluent was taken for preliminary testing.

The initial sample mentioned above was collected approximately 20 days before our first test, and was stored at room temperature (approximately 25°C) during this time. It was noted that the yellow precipitate originally observed in the effluent had darkened and become black by the time of the test. The presence of precipitate in later samples, as well as the color of the suspended material, varied both between production platforms and with data of collection (Table 1).

PRELIMINARY TESTING

Preliminary testing began in July 1977 with experiments to define the limits of toxicity of the effluent. In this series of tests Buccaneer produced brine effluent was tested at concentrations of 500,000; 100,000; 10,000; 1,000 and 1 ppm (volume of effluent/volume of diluent). Although salinity of the brine (35.5 o/oo) tested was within limits usually tolerated by these animals, to ensure that salinity change was not a cause of mortality, natural sea water obtained from the laboratory system filtered through a 5-u filter passed over an ultraviolet light was brought to the same salinity with concentrated sea water and then used as test medium at 500,000 and 100,000 ppm.

As a further test, both salinity-adjusted sea water and brine effluent were treated with Buccaneer Oilfield petroleum hydrocarbons. Four parts sea water or effluent were mixed with one part petroleum and stirred on a magnetic stirrer for 10 minutes. The resulting emulsions were placed in spearatory funnels, allowed to stand for 30 minutes, and the aqueous layers drained off and diluted for testing. Petroleum treated brine was tested at 500,000 and 100,000 ppm; salinity-adjusted sea water (as described above) at 500,000, 100,000 and 10,000 ppm (Table 2).

All effluents and test solutions as described were diluted with natural sea water aerated for 2-3 days. Salinity of this water was 25 o/oo, and was identical to that in which the experimental animals were maintained.

The experimental white shrimp were hatched and reared in this laboratory; at the date of the range-finding experiment, test animals averaged 28.26 mm in total length (from tip of rostrum to tip of telson) and ranged from 21-34.5 mm. Five animals were placed in 500 ml test medium contained in a 1000 ml glass beaker, with two beakers (10 animals) per test solution. Medium was neither aerated nor changed during the test, nor were animals fed.

In the first stage of the test, white shrimp (P. setiferus) were exposed to 500,000 ppm effluent; 500,000 ppm effluent saturated with petroleum; 100,000 ppm effluent; 100,000 ppm effluent saturated with petroleum; 500,000 ppm salinity-adjusted sea water; 500,000 ppm salinity-adjusted sea water saturated with petroleum; and natural sea water alone (0 ppm effluent) as a control.

Within 10 minutes of exposure, animals subjected to both 500,000 ppm petroleum-saturated effluent and salinity-adjusted sea water were

moribund; i.e., they were lying on their sides with no indication of pleopod motion unless stiumlated with a glass rod. All animals in these solutions were dead (no response to stimulation with glass rod; no visible heart beat) within 45 minutes of exposure. Animals in 500,000 ppm effluent showed immediate stress (hyperactivity; erratic swimming movements) within 10 minutes, and all were dead within 3 hours. Animals exposed to 100,000 ppm effluent showed no signs of stress during the first 4 hours, but were dead within 20.5 hours, while animals in the 100,000 effluent saturated with petroleum showed immediate stress, with 20% moribund within 45 minutes. Two deaths (20%) occurred within 4 hours. All of these animals had also died 20.5 hours after exposure. Of this group of experimental animals, only those exposed to 500,000 ppm salinity-adjusted water and animals in natural sea water survived 24 hours of observation (Table 2).

Because test concentrations as described were too high to permit 96-hour survival, the test was repeated using effluent concentrations of 10,000, 1,000 and 1 ppm. Salinity-adjusted water had been shown to be virtually non-toxic at 500,000 ppm thereby eliminating salinity change as cause of stress and death; therefore, lower concentrations of this water were not tested. To further test the effect of hydrocarbons, petroleum-saturated salinity-adjusted water was tested at 100,000 and 10,000 ppm. As contrasted with the animals in the range-finding experiment, no signs of stress were noted during the first 4 hours among any animals exposed in the second test. Seventy per cent of the animals exposed to 10,000 ppm effluent were dead 20.5 hours after exposure, but no more than 20% of the animals exposed to any of the other conditions were affected. Of the six animals that died at the lower concentrations, five died following a molt

and had been partially eaten by other animals in the beaker. At the end of 96-hours, during which the water was not changed or aerated, survival was 80-90% under all test conditions except 10,000 ppm effluent which had no survival and natural sea water which had 100% survival. This experiment established that 10,000 ppm was the upper limit at which concentrations of effluent needed to be tested, and suggested that the addition of petroleum to brine effluents made it more toxic than petroleum in natural sea water.

96-HOUR BIOASSAYS

Methods

Static bioassays were conducted according to the general methods outlined by the EPA (1976). Ten animals were used per test concentration, the number per beaker varying with the size of the animal and series (Table 3). One liter glass beakers containing 500 ml test medium were used. Beginning in September, test solution was replaced daily to ensure more constant levels of exposure to effluent and adequate oxygen supply, and to reduce the possibility of death from accumulation of toxic metabolites.

All test media (dilutions) were prepared using sea water from the laboratory circulating system, aerated for several days prior to test. Experimental beakers were covered with 6 mil polyethylene film, both to prevent test animals from jumping out of the beaker while in stress and to reduce evaporation with consequent loss of test material and change in salinity.

All experimental animals, both white and brown shrimp, were hatched in the laboratory and maintained at salinities of 25-33 o/oo and

temperatures ranging from 23-26°C. Experiments were conducted in controlled temperature rooms at 25°C±1°C and salinities of 25 0/00. Test concentrations ranged from 1,000 to 50,000 ppm (Table 4), based upon the preliminary range-finding experiment.

Beginning in November, oxygen concentrations of the media were measured daily just before the medium was replaced. A Yellow Springs Instrument Model 57 oxygen meter was used. Measurements were made only in beakers in which shrimp survived, since bacterial decomposition could not be eliminated as major contributions to oxygen depletion in the presence of dead shrimp.

Results

Toxicity of brine effluent varied slightly with date of collection, collection site, and species (Table 3). The most marked change in toxicity appeared in the sample collected in October at which time no biocide was being added to the effluent from Platform 288-A (personal communication, Mr. J. A. Burgbacher, Shell Oil Company). Concentrations of 10,000 ppm effluent from Platform 288-A permitted survival of 70% of the small and 60% of the larger shrimp (61.1 mm average length) tested (Table 4). Effluent from Platform 296-B retained its toxicity with 25% of the smaller animals and 20% of the larger ones surviving 10,000 ppm.

Critical concentrations for the brine effluent collected in 1977 seemed to fall between 5,000 and 10,000 ppm (except the October sample), and were between 1,000 and 5,000 ppm for the two samples collected in January and February, 1978. Hydrocarbon and trace metal analyses of the effluents have not been available for comparison with survival data. Also, no biocides were being added to the produced brine when the October sample was taken (see above).

Oxygen concentration measurements indicated that there was a significant increase in average daily oxygen consumption with time of exposure (P $\leq$ than 0.01), but no significant difference occurred in oxygen consumption between control animals and those in effluent concentrations too low to cause mortality (Table 5).

LONG-TERM EFFECTS

Methods

Groups of ten white shrimp (50.2 mm mean length) were placed in each of six glass aquaria, 20 x 40 x 20 cm, fitted with subsurface filters, and containing oyster shell and sand substrate (Zein-Eldin and Aldrich, 1965) and 14 l of natural sea water. All groups were given a prepared feed (Fenucci and Zein-Eldin, 1976), first weighed, then soaked 30 minutes in one of three media: natural sea water; brine effluent (obtained November 14, 1978); and brine effluent (obtained December 22, 1977). Two groups of shrimp, selected at random, were given each treatment. All groups were fed at the same rate, 0.5 g dry feed twice each day. Feeding was continued for 42 days, which included at least two complete molt cycles of the surviving shrimp.

Results

Animals given effluent-soaked feed survived and grew as well as animals given feed soaked in natural sea water. Response to the presence of feed appeared to be as rapid among animals given the effluent-soaked feed as among the controls. Molting oxcurred among all groups, with no apparent difference between test media. Survival was approximately the same among all groups: 55% for control shrimp (feed soaked in sea water), 60% among animals given feed soaked in effluent collected November 4, and 65% among animals given feed soaked in effluent collected December 22.

Surviving animals were maintained in the aquaria for an additional 14 days, and were fed unsoaked, untreated dry feed. At the end of this period, half of each group was challenged in a 96-hour tolerance test using 5000 ppm of effluent obtained January 29, 1978 (Table 6). Both survival and oxygen consumption differed between control animals subjected only to the 96-hour bioassay and those previously held and exposed to effluent-soaked feed. Those animals that previously ingested effluent-soaked feed used more oxygen daily when further stressed with 5,000 ppm effluents than did animals previously ingesting effluent-soaked feed but exposed only to nautral sea water during the 96-hour test. Differences in oxygen consumption between animals in natural sea water and in effluent continued throughout the 96-hours for animals that previously ingested effluent-soaked feed, but were not significant either after 72 or 96 hours among animals previously ingesting only sea water-soaked feed, nor among those animals in the simultaneous 96-hour bioassay using the same effluent (Table 7).

It appears that long-term exposure of shrimp to effluent-soaked feed may predispose them to exhibit stress upon additional exposure to test media, whether in the form of increase in oxygen consumption or in actual death. It is of interest, however, that both groups of animals that were held in small aquaria (Table 6) for approximately 8 weeks prior to the bioassay showed lower mortality rates than did those confined for the first time at the start of the bioassay. Whether this is an adaptation to reduced container size exhibited by the animals given feed containing effluents remains to be studied.

FACTORS AFFECTING THE BIOASSAY

Biological

In crustaceans, the exoskeleton is replaced periodically through molting, premitting growth of the animal. The process is cyclic, the duration of the cycle varying with temperature, size and other characteristics of the animal, and species. Although it is possible to determine the stage of the molt cycle in indivisual animals (Schafer, 1968) it requires considerable handling of each animal, a procedure which increases stress. To prevent the additional stress prior to the addition of test media, animals were randomly assigned to experimental treatments without regard to pre-molting stage.

It was inevitable that some animals would molt during the 96-hour bioassays, with two resulting problems: (1) some newly molted animals were eaten by others when two or more animals were placed in a beaker; and (2) oxygen level in the medium was reduced because of the additional oxygen required by the animal during the molting process (Zein-Eldin, 1960). Observed molting varied among the experiments, with a single molt found in the test of July 18 (Table 8) and 37 molts in the series of October 31 (effluent collected October 28). Therefore, survival following molting was greater in the later experiments in which only one or two animals were placed in a beaker (Table 8). Of 27 animals molting during the first three series (including the preliminary range-finding experiment), only 5 survived, for a survival rate of 18% among animals molting in beakers containing 5 animals. Among groups containing 2 animals, 25 individuals survived 46 molts (54%), but 51 animals of those placed one per container survived 72 molts (61%).

Because of the dependence between number of animals in the container and their survival following the stress of molting, it is strongly recommended that single animals be used in test containers, with appropriate replication, when juvenile penaeids (25 mm or greater total length) are to be used as bioassay organisms.

Physical Characteristics of the Effluent

There was some indication that the suspended matter in the brine effluent was more toxic than the shole effluent (Table 9). Thus, only supernatant fluid was used in the second series in which effluent collected on June 26 was retested. In this experiment 60% of the animals survived 10,000 ppm effluent, although no animals had survival the addition of 10,000 ppm whole effluent only one week earlier.

To test the hypothesis of greater toxicity of the whole effluent, one further test was made on September 1, again using the June 26 effluent which had been stored at room temperature (^{o}C- Table 9). It is apparent that there were no significant differences in effect between the whole brine and the supernate; however, true effects may well have been confounded both by the use of 5 animals per beaker and by the long period of storage. More definitive experiments should be conducted to separate suspended material by either centrifugation of filtration, then resuspending it at known concentrations in natural sea water for toxicity comparisons against both supernatant fluid alone and whole effluent.

Addition of Petroleum Hydrocarbons to the Brine Effluent

The addition of petroleuy hydrocarbons brought about a dramatic decrease in the time until death at the higher concentrations tested (Table 2), as noted particularly in the preliminary test. Subsequent

experiments yielded less certain results (Table 4), particularly in the experimental series in which 5 animals were placed in each test beaker. A picture more like that of the preliminary test occurred in the test of the effluent collected on August 21 with larger white shrimp (P. setiferus) (Table 10). In this experiment mortality was both higher and occurred more rapidly in the presence of added petroleum than in the same effluent without additional petroleum. Experiments with petroleum addition were discontinued when we began daily water changes because of the limited amount of effluent available.

DISCUSSION

LD_{50}'s reported here for penaeid shrimp are much higher than those reported using various crude oil mixtures (Heitz et al., 1974; Tatem, Cox, and Anderson, 1978). The important difference is that our tests have been conducted using actual discharge fluids rather than with purified components and were performed in natural sea water, not in artificial sea waters used in the other references cited above.

Heitz et al. (1974) state that the level of oil toxicity is related to season and/or salinity, but the previous handling of their animals which were obtained from nature and subjected to several changes in both salinity and temperature prior to test make these statements difficult to assess. Unlike Tatem, Cox and Anderson (1978) who acclimated the animals for 7-14 days prior to test, Heitz et al. (1974) made no mention of acclimation prior to testing, nor do they discuss the procedure for emulsifying the oil dispersions. Both authors reported LD_{50}

values of 2 to 35 ppm, considerably lower than those determined using the Buccaneer brine effluent mixed with petroleum as described.

The long-term tests suggested that exposure to ingested effluents may well predispose penaeids to stress in subsequent acute exposure tests. Middleditch, Basile and Chang (1978) reported the presence of petroleum hydrocarbons in brown shrimp (P. aztecus) obtained from areas near the Buccaneer Oilfield production platforms. Brown shrimp were abundant as postlarvae (Harper, 1978) and juveniles (Emiliani et al., 1978) in the area. It is thus possible for penaeids to be exposed to contaminants in sea water, sediments or food items in the area.

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APPENDIX

TABLE 1. Source and physical characteristics of produced brine effluents tested.

SAMPLE COLLECTION DATE	PRODUCTION PLATFORM	COLOR AND PHYSICAL CHARACTERISTICS	SALINITY (o/oo)
06/26/77	296-B	Yellow; black when tested	36
07/15/77	288-A	Yellow; some suspended particles	48
	296-B	Yellow; some suspended particles	35
08/21/77	288-A	Black; turbid	51
	296-B	Slightly yellow; turbid	36
09/18/77	288-A	Black; turbid	32
	296-B	Clear; with yellow particles	32
10/28/77	288-A	Yellow; clear	42
	296-B	Black; turbid	40
11/14/77	296-B	Yellow; slightly turbid	42
12/22/77	296-B	Grey; slightly turbid	41
01/29/78	296-B	Yellow; slightly turbid	40
02/28/78	296-B	Grey; turbid	42

TABLE 2. Range finding bioassay with produced brine effluent collected on June 26, 1977. Test were conducted July 12, 1977 except where otherwise noted.

CONCENTRATION (ppm, V/V)[a]	UNTREATED EFFLUENT		UNTREATED SALINITY ADJUSTED		PETROLEUM TREATED EFFLUENT		PETROLEUM TREATED SALINITY-ADJUSTED	
	Time to 100% Mortality, Hours	96-Hour Survival %	96-Hour Survival %	Time to 100% Mortality, Hours	96-Hour Survival %	Time to 100% Mortality Hours	96-Hour Survival %
0	[b]	100	-	1.5[c]	0		
1	-	80	-	-	-	-	-
1000	-	90	-	-	70[c]		
10,000	>24 <29	0	-	-	50[c]	-	80
100,000	>5 <21	0	-	>3 <20	0	-	70
500,000	<3	0	60[d]	.75	0	.75	0

[a] Aerated natural seawater used to dilute brine

[b] Not tested

[c] Tested 07/18/76

[d] Two shrimp molted and were eaten

TABLE 3. Summary of experimental protocol and results of bioassays of Buccaneer Oilfield produced brine effluents.

SAMPLE COLLECTION DATE	PRODUCTION PLATFORM	SPECIES	NO. SPECIMENS PER CONTAINER	NO. OF CONTAINERS	MEAN SIZE TOTAL LENGTH (mm)	LD_{50} (ppm)
06/26/77	296-B	white shrimp (*P. setiferus*)	5	2	28.3	3000
07/15/77	288-A	white shrimp (*P. setiferus*)	5	2	29.8	3500
	296-B	white shrimp (*P. setiferus*)	5	2	29.8	3500
08/21/77	288-A	white shrimp (*P. setiferus*)	5	2	29.6	1000[a]
			2	5	48.2	4500[a]
	296-B	white shrimp (*P. setiferus*)	5	2	29.6	1000[a]
			2	5	48.2	6000[a]
09/18/77	288-A	white shrimp (*P. setiferus*)	1	10	59.3	6000
		brown shrimp (*P. aztecus*)	1	10	55.2	3800
	296-B	white shrimp (*P. setiferus*)	1	10	59.3	6500
		brown shrimp (*P. aztecus*)	1	10	55.2	6000
10/28/77	288-A[b]	white shrimp (*P. setiferus*)	2	10	37.1	>10,000
			1	10	61.1	>10,000
	296-B[b]	white shrimp (*P. setiferus*)	2	10	37.1	7000
			1	10	61.1	5000

(Cont'd)

TABLE 3. Summary of experimental protocol and results of bioassays of Buccaneer Oilfield produced brine effluents.

SAMPLE COLLECTION DATE	PRODUCTION PLATFORM	SPECIES	NO. SPECIMENS PER CONTAINER	NO. OF CONTAINERS	MEAN SIZE TOTAL LENGTH (mm)	LD_{50} (ppm)
11/14/77	296-B	white shrimp (*P. setiferus*)	1	10	58.7	6500
12/22/77	296-B	white shrimp (*P. setiferus*)	1	10	58.7	5000
01/29/78	296-B	white shrimp (*P. setiferus*)	1	10	64.4	1750
02/28/78	296-B	white shrimp (*P. setiferus*)	1	10	64.4	2100

[a] Control (0 ppm) survival for 96-hours was very poor; these values are given for 24-hours only.

[b] No biocides were being added when this sample was collected.

TABLE 4. 96-hour survival (%) of penaeid shrimp exposed to various concentrations of Buccaneer Oilfield produced brine effluents. White shrimp (Penaeus setiferus) used as test animals unless otherwise indicated (refer to Table 3 for shrimp size).

Sample Collection Date	Production Platform	Control 0 %	Effluent 1000 %	Petroleum Treated 1000	5000 %	Effluent 10,000 %	Petroleum Treated 10,000	50,000 %	100,000 %
07/15/77	288-A	70	90	100	-[b]	10	10	-	-
	296-B	70	70	70	-	20	0	-	-
08/21/77	288-A	45	30	60	-	0	0	-	-
	288-A	20	50	30	-	0	0	-	-
	296-B	45	30	70	-	20	50	-	-
	296-B	20	46	50	-	10	0	-	-
09/18/77	288-A	100	80	-	70	0	-	-	-
	A[c]	90	80	-	40	0	-	-	-
	296-B	100	100	-	80	0	-	-	-
	296-B[c]	90	80	-	80	0	-	-	-
10/28/77	A	90	85	-	75	70	-	-	-
	A	100	90	-	90	60	-	-	-
	296-B	90	90	-	75	25	-	-	-
	296-B	100	90	-	50	20	-	-	-
11/14/77	296-B	80	-	-	80	0	-	0	0
12/22/77	296-B	80	-	-	50	0	-	0	0
01/29/78	296-B	90	60	-	30	0	-	-	-
02/28/78	296-B	90	90	-	0	0	-	-	-

[a] See text for explanation

[b] Concentration not tested

[c] Penaeus aztecus

TABLE 5. Oxygen concentration (ppm) in the medium of white shrimp (P. setiferus) exposed to Buccaneer Oilfield produced brine effluents during a 96-hour bioassay.[a]

TEST DATE	SAMPLE COLLECTION DATE	BRINE EFFLUENT CONCENTRATION (ppm)	24 Hours		48 Hours		72 Hours		96-Hours	
			No. Shrimp	O_2 ppm*	No. Shrimp	O_2 ppm*	No. Shrimp	O_2 ppm*	No. Shrimp	O_2 ppm*
01/17/78		0	10	2.54±0.55	9	1.90±0.54	9	2.71±0.49	6	3.39±-.87
	11/17/78	5000	10	2.71±0.67	9	2.72±0.62	9	2.96±0.82	6	3.72±0.87
	12/22/77	5000	10	2.56±0.75	9	2.11±0.78	5	3.39±0.63	5	3.62±0.82
03/06/78		0	19	1.98±0.41	20	2.05±0.79	19	2.76±1.09	18	3.33±0.74
	01/29/78	5000	10	2.40±0.83	8	1.92±0.72	8	3.01±0.69	6	3.00±0.74
	02/28/78	5000	10	1.77±0.58	9	2.12±0.75	9	2.81±0.76	9	2.62±0.95

[a]Data from October sample not included; oxygen measurements were taken every other day.
*Mean ± standatd deviation; includes only those measurements made on media containing a single live animal that had not molted during the 24-hour period. Medium changed after oxygen measurement.

TABLE 6. 96-hour survival (%) of white shrimp given produced brine effluent-soaked feed for 42 days prior to exposure to produced brine collected January 1978.

PREVIOUS CONTAINER SIZE	PREVIOUS TREATMENT	CONCENTRATION OF EFFLUENT	
		0 % ppm	5,000 % ppm
20 x 40 x 20 cm	Feed soaked in seawater	80	83
20 x 40 x 20 cm	Feed soaked in produced brine effluent	100	54
180 cm diam. x 40 cm	No acclimation; unsoaked dried feed	90	30

TABLE 7. Oxygen concentration in the medium during a 96-hour bioassay of white shrimp (P. setiferus) previously exposed to feed soaked in produced brine effluents.

PREVIOUS TREATMENT	BRINE EFFLUENT CONCENTRATION (ppm)	24 Hours		48 Hours		72 Hours		96 Hours	
		No. Shrimp	O_2 ppm	No. Shrimp	O_2 ppm	No. Shrimp	O_2 ppm	No. Shrimp	O_2 ppm
Regular Bioassay	0	19	1.98±0.41*	20	2.05±0.79*	19	2.76±1.09*	18	3.33±0.74*
	5000	10	2.40±0.83	8	1.92±0.72	8	3.01±0.69	6	3.00±0.74
Sea Water Soaked Feed	0	4	2.04±0.66	4	1.51±0.22	4	2.90±0.46	4	2.62±0.77
	5000	6	1.30±0.65	6	2.17±0.87	5	2.98±1.04	5	2.81±1.27
Effluent Soaked Feed	0	12	1.88±0.58	12	2,52±1,14	12	3,05±0.88	11	3.38±0.66
	5000	10	1.26±0.22	10	2.16±0.87	8	1.86±0,87	7	2.77±0.99

*Mean ± standard deviation.

TABLE 8. Relationship of molting mortality to number containers (beakers) produced brine effluent bioassays.

SAMPLE COLLECTION DATE	NUMBER OF SPECIMENS PER CONTAINER	NUMBER OF MOLTS	DEATHS AFTER MOLT	% MOLTING DEATHS
06/26/77	5	8	8	100
07/15/77	5	1	0	0
08/21/77	5	18	14	78
TOTAL		27	22	81
08/21/77	2	9	7	78
10/28/77	2	37	14	38
TOTAL		46	21	46
09/18/77	1[a]	21	2	10
09/18/77	1[a]	19	7	37
10/28/77	1	22	9	41
11/14/77	1	6	2	33
12/22/77	1	0	0	0
01/29/78	1	2	0	0
02/28/78	1	2	1	50
TOTAL		72	21	29

[a]Brown shrimp (P. aztecus)

TABLE 9. Percent survival (%) of penaeids (species combined) exposed to supernatant or whole effluent collected 06/26/77 and stored at room temperature.

DATE OF TEST		CONCENTRATION, ppm 1,000 %	10,000 %
07/12/77	Mixed brine effluent	90	0
09/01/77	Mixed brine effluent	40	40
07/18/77	Supernatant	70	60
09/01/77	Supernatant	50	30

TABLE 10. Survival of penaeids (species combined) exposed to brine effluent or petroleum-treated produced brine effluent August 21, 1977.

UNTREATED

PRODUCTION PLATFORM	CONCENTRATION ppm	TIME TO 100% MORTALITY HOURS	96-HOUR SURVIVAL %
288-A	1,000	--	50
	10,000	>24 <44	0
296-B	1,000	--	46
	10,000	--	10

PETROLEUM-TREATED

PRODUCTION PLATFORM	CONCENTRATION ppm	TIME TO 100% MORTALITY HOURS	96-HOUR SURVIVAL %
288-A	1,000	--	30
	10,000	>12 <20	0
296-B	1,000	--	50
	10,000	> 2 <24	0